2009. The Journal of Arachnology 37:368–370

SHORT COMMUNICATION

Reducing scorpion fluorescence via prolonged exposure to ultraviolet light

Carl T. Kloock: Department of Biology, California State University, Bakersfield, 9001 Stockdale Highway, Bakersfield, California, 93311, USA. E-mail: ckloock@csub.edu

Abstract. A simple technique is presented for reducing the fluorescence of living scorpions by prolonged exposure to UV light. Scorpion's fluorescence peak can be eliminated by a 1-mo exposure to low intensity UV light. Although the fluorescence peak returns within 1 wk after removal from UV light exposure, the magnitude remains reduced. This technique potentially opens up new options for testing a variety of hypotheses about possible functions of scorpion fluorescence including potential effects on cuticle strength, visual responses, predation, cannibalism, and mating.

Keywords: Spectra, spectroscopy

There is no known function of scorpion fluorescence (Brownell 2001; Kloock 2008). Although it is certainly possible that fluorescence has no function, it is only by testing and falsifying potential functions that they can be eliminated from consideration. In order to test potential functions of scorpion fluorescence, having scorpions with reduced fluorescence could be a powerful tool. Several methods for reducing fluorescence exist, but those currently available are problematic. Kloock (2005) removed fluorescence from preserved scorpions by applying a varnish that blocked ultra-violet light (UV), and fluorescence can also be eliminated or reduced either by not supplying UV light or blocking it with filters. Use of coatings limits experiments because live, non-fluorescing scorpions cannot be used. In addition, coating scorpions introduces experimental complications, given the different chemicals needed to either block or allow fluorescence and secondary coatings to remove these effects dim the fluorescence of controls. Detecting fluorescence under natural conditions, if possible, requires very sensitive visual senses that scorpions may possess (Kloock 2008), but any dimming could prevent detection, and thus affect experimental attempts to demonstrate detection. Eliminating or blocking UV light with filters prevents fluorescence of living specimens, but also introduces a necessary experimental complication: effects of fluorescence and UV light cannot be separated by techniques that simply eliminate UV light. Given that scorpion eyes (Machan 1968) and their extra-ocular light sense (Zwicky 1970) are sensitive to both UV light and light near the fluorescence peak (~500 nm), this is a serious complication. Modifying scorpions so that they can be exposed to UV light without fluorescing can remove this problem, although possible behavioral and physiological side effects of any such manipulation could introduce new difficulties.

Anecdotal accounts suggest that long-term exposure to ultraviolet radiation may reduce scorpion fluorescence (Wankhede 2004). This has not previously been quantitatively demonstrated. I present here evidence that long-term exposure to UV light significantly reduces scorpion fluorescence.

Paruroctonus becki (Gertsch & Allred 1965) (Vaejovidae) were collected July–September 2008 in Kern County, California (voucher specimens deposited at the California Academy of Sciences), and housed in small, foam-plugged plastic vials. Sixteen females were randomly chosen from this population for the experimental manipulation and placed in small open-topped plastic arenas (13 cm long × 10 cm wide × 7 cm high) with 50 ml of soil collected from the same site as the scorpions. The soil helped to absorb and retain moisture, but was not deep enough to allow scorpions to bury themselves to escape irradiation. Scorpions were exposed 24 hrs/day for 32 days to two 40 W fluorescent blacklights (GE F40BLB) at a constant distance of 75 cm. This resulted in scorpions constantly receiving 11 $\mu W/cm^2$ of UV light energy (measured with a Mannix UV-340 light meter: range = 290–390 nm). Control scorpions were kept without significant UV light exposure. They were exposed to light from white fluorescent lights (≤ 1 $\mu W/cm^2$ UV) daily. Once a week, each scorpion was fed a single mealworm larva (*Tenebrio* sp.) and provided water by spraying the soil surface with a mister. I selected only females to simplify analysis and because females were more readily available. I see no reason to expect major differences between genders, but this should be tested in the future.

Within 2 wk, UV-exposed scorpions exhibited visibly reduced fluorescence, particularly on the dorsal surfaces. The thinner, more flexible portions of the exoskeleton (i.e., carapace and mesosoma) experienced a larger fluorescence reduction than the thicker regions (pedipalps, metasoma). After 32 days, fluorescence on the dorsal surfaces was no longer visible, though ventral surfaces and chelicerae still fluoresced dimly, in a pattern consistent with the effects of shading. At this point, the 12 surviving UV-exposed scorpions and 12 randomly selected control scorpions were measured with a reflectance spectrometer using a UV light source to stimulate fluorescence.

Emission spectra measurements were taken with a BW-Tek BRC111A CCD Spectrometer using BWSpec version 2.24 software with an integration time of 100 ms and 20 averages per sample. A single UV LED with peak emission at 390 nm and transmitted via a fiber-optic cable directly to the reflectance probe supplied excitation energy. A light-absorbing fabric (Edmund scientific #3060068) served as a dark reference, and I used the dark subtraction method for all measurements. The spectra were analyzed in raw form, without smoothing techniques (e. g., Fourier or running averages) applied to remove noise from the spectra.

All spectrum measurements occurred inside a light-tight box enclosing both the scorpion and reflectance probe. A squeeze cage, as described in Kloock (2008), immobilized scorpions during measurement. The reflectance probe was lowered until it touched the carapace at a point just behind the medial eyes. The order of measurements was randomized to reduce potential bias.

Fig. 1 provides a visual demonstration of the results of the manipulation. More importantly, spectrometer measurements showed large effects (Fig. 2, Table 1). The peak seen in each spectrum at ~400 nm (Fig. 2) results from reflection of the UV light source (peak emission at 390 nm) and is not the result of fluorescence. To avoid this artifact, all measurements were made over the range of 450–700 nm.

Thirty-two days of UV exposure produced a significant reduction in peak power (Table 1; *t*-test assuming unequal variance, $t = 8.88$, $P = 2.39 \times 10^{-6}$, $df = 12$) and a significant increase in the wavelength

Figure 1.—Photograph (grayscale) of two scorpions under black-light against a non-fluorescent white background. The scorpion on the left received prolonged exposure to UV light, the scorpion on the right did not. Preserved specimens were photographed due to the long exposure times required under these lighting conditions. Photo courtesy of Jason-Marc Mohamed. Used with permission.

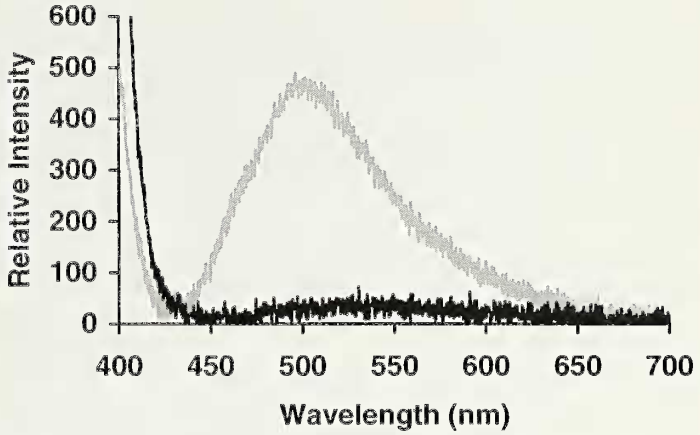

Figure 2.—Representative spectra in the visible range (400–700 nm) of a control scorpion (gray line) and a scorpion after 32 days of exposure to 11 μW/cm^2 UV light (black line). The intensity peak in both spectra as they approach 400 nm is eaused by refleetion from the light source, an UV LED with peak emission at 390 nm.

of peak power (*t*-test assuming unequal variance, $t = 4.48$, $P = 0.0007$, $df = 12$). The emission spectra for the UV-exposed scorpions show no clear peaks (Fig. 2). An *F*-test for variance ratios (Zar 1996) shows that the variance in the peak wavelength of the UV-exposed scorpions far exceeded the variance for control scorpions (UV-exposed scorpions $s^2 = 655$, control scorpions $s^2 = 19.0$, $F = 34.5$, $P = 6.46*10^{-7}$). This and the wide range of values (Table 1) indicate that the wavelength of peak power was essentially random for the UV-exposed scorpions.

After measurement, both UV exposed and control scorpions were placed in covered containers with soil and a shelter (a fragment of terra cotta pot) and maintained on a 13 hours white light: 11 hours dark cyclc, with feeding and watering schedules maintained as above. One week later, spectra were remeasured. Table 1 shows that in one week without UV exposure, significant recovery of fluorescence had occurred, with consistent peaks again evident in the spectra and no difference between groups in wavelength of peak power (*t*-test assuming unequal variance, $t = 1.32$, $P = 0.204$, $df = 12$). Although the relative intensity of the fluorescence was still significantly reduced compared with controls (*t*-test assuming unequal variance, $t = 4.60$, $P = 6.06 \times 10^{-4}$, $df = 12$.), fluorescence intensity increased significantly within the UV-exposed group over the one-week recovery period (*t*-test assuming unequal variance, $t = 3.56$, $P = 1.56 \times 10^{-4}$, $df = 12$). Significant differences in the variance between controls and UV-exposed scorpions still existed for peak intensity ($F = 35.2$, $P = 5.79 \times 10^{-7}$) and for peak wavelength ($F = 3.28$, $P = 0.030$). Preserved UV-reduced specimens showed no signs of recovery, indicating that active metabolic processes are responsible for recovery. Determining just what those proccsses are will require further study.

Photobleaching, the loss of fluorescence due to prolonged exposure to excitation wavelengths, is commonly encountered in fluorescence microscopy, which focuses on methods of preventing it (Deschenes & Vanden Bout 2002). Photobleaching is probably caused by a variety of different mechanisms (Georgakoudi et al. 1997), so more detailed study will be needed to determine a mechanism of the photobleaching observed here. Two molecules responsible for scorpion fluorescence have been identified: ß-carbolinc (Staehel et al. 1999) and 4-methyl-7-hydroxycoumarin (Frost et al. 2001), which may aid future investigations into this phenomenon. Similarly, the mechanism of recovery has not yet been investigated, and possible side effects of the treatment, including the potential of rctinal or other tissue damage and effects on behavior of long-term exposure, need to be investigated and controlled in any future experiments using this technique.

With this important caveat, the ability to reduce fluorescence potentially opens up a broad variety of new experiments. For example, Camp & Gaffin (1999) and Blass & Gaffin (2008) suggest that fluorescence may function as a light amplifier, or aid in detecting UV light. If true, then scorpions with reduced fluorescence should display an altered light avoidance response. Tests can also be designed to test ecological hypotheses of function (summarized in Kloock 2008). For example, experiments could be designed to test whether scorpions with reduced fluorcseenee experienee different levels of predation, cannibalism, prey capture or mating success than fluorescent scorpions under diffcrent lighting conditions in both natural and laboratory settings.

It is also possible that fluoreseence is a byproduct of a molecule whose primary function is unrelated to fluorescenee itself. For example, Stachel et al. (1999) suggested that the fluorescent molecule functions in sclerotization. To test this hypothesis, experiments can be designed to test the effects of fluorescence reduction on cuticle strength. Similarly, the fluorescent molecule may function in reducing water loss (Lourenço & Cloudsley-Thompson 1996), and experiments could be designed to determine the effect of fluorescence reduction on the rate of water loss.

Caution must be exercised in experimental design because we have, as yet, no information on side effects of the technique of fluorescence

Table 1.—Data from fluorescence spectra of control and UV-exposed scorpions after 1 mo of exposure, and repeated after 1 wk of recovery. $n = 12$ for each treatment. Peak values determined over the range 450–700 nm.

Measurement	Treatment	Mcan peak relative intensity (SE)	Mean wavelength of peak intensity, nm (SE)	Range: wavelength of pcak intensity, nm
After exposure	Control	608 (120)	501 (1.26)	496–511
	UV-exposed	72.1 (10.2)	535 (7.39)	497–576
After recovery	Control	482 (77.2)	501 (2.16)	492–519
	UV-exposed	122 (13.0)	508 (3.91)	490–533

reduction itself on scorpion behavior or physiology, which could complicate future experiments. The challenge of experimental design using this technique will be to adequately control for potential side effects of fluorescence reduction. One way to accomplish this will be to cross UV presence and fluorescence reduction: if fluorescence reduction has an effect unrelated to fluorescence, similar differences should be observed between fluorescence-reduced and control scorpions regardless of the presence of UV light. Of course, the details of any experiment may require more complicated controls to be developed. Provided adequate controls are used, this technique makes possible future experiments designed to determine whether or not scorpion fluorescence (or the fluorescent molecules) serves specific functions. Even if fluorescence serves no function, such experiments can enhance our understanding of scorpion ecology, physiology, and behavior.

ACKNOWLEDGMENTS

A CSUB University Research Council Grant supplied funding. J. Mohamed provided the photograph and Dr. P. Mickler allowed use of his photographic equipment. I thank the reviewers for their comments, which improved the manuscript.

LITERATURE CITED

Blass, G.R.C. & D.D. Gaffin. 2008. Light wavelength biases of scorpions. Animal Behaviour 76:365–373.

Brownell, P. 2001. Sensory ecology and orientational behaviors. Pp. 159–183. *In* Scorpion Biology and Research. (P. Brownell & G.A. Polis, eds.). Oxford University Press, Oxford, UK.

Camp, E.A. & D.D. Gaffin. 1999. Escape behavior mediated by negative phototaxis in the scorpion *Paruroctonus utahensis* (Scorpiones, Vaejovidae). Journal of Arachnology 27:679–674.

Deschenes, L.A. & D.A. Vanden Bout. 2002. Single molecule photobleaching: increasing photon yield and survival time through suppression of two-step photolysis. Chemical Physical Letters 365:387–395.

Frost, L.M., D.R. Butler, D. O'Dell & V. Fet. 2001. A coumarin as a fluorescent compound in scorpion cuticle. Pp. 365–368. *In* Scorpions 2001: in Memoriam, Gary A. Polis. (V. Fet & P.A. Seldon, eds.). British Arachnological Society, Burnham Beeches, Buckinghamshire, UK.

Gertsch, W.J. & D.M. Allred. 1965. Scorpions of the Nevada test site. Brigham Young University Science Bulletin Biological Series 6:1–15.

Georgakoudi, I., M.G. Nichols & T.H. Foster. 1997. The mechanism of Photofrins® photobleaching and its consequences for photodynamic dosimetry. Photochemistry and Photobiology 65:135–144.

Kloock, C.T. 2005. Aerial insects avoid fluorescing scorpions. Euscorpius 21:1–7.

Kloock, C.T. 2008. A comparison of fluorescence in two sympatric scorpion species. Journal of Photochemistry and Photobiology B: Biology 91:132–136.

Lourenço, W.R. & J.L. Cloudsley-Thompson. 1996. The evolutionary significance of colour, colour patterns and fluorescence in scorpions. Revue Suisse de Zoologie, Vol., hor série II: 449–458.

Machan, L. 1968. Spectral sensitivity of scorpion eyes and the possible role of shielding pigment effect. Journal of Experimental Biology 49:95–105.

Stachel, S.J., S.A. Stockwell & D.A. Van Vranken. 1999. The fluorescence of scorpions and cataractogenesis. Chemistry and Biology 6:531–539.

Wankhede, R.A. 2004. Extraction, isolation, identification and distribution of soluble fluorescent compounds from the cuticle of scorpion (*Hadrurus arizonensis*). M.S. Thesis, Marshall University, Huntington, West Virginia. 61 pp.

Zar, J.H. 1996. Biostatistical Analysis, 3rd edition. Prentice Hall, Upper Saddle River, New Jersey. 662 pp.

Zwicky, K.T. 1970. The spectral sensitivity of the tail of *Urodacus*, a scorpion. Specialia 15:317.

Manuscript received 15 October 2008, revised 1 April 2009.